

72 Repeated Eradications of *Pseudomonas aeruginosa* in Cystic Fibrosis Patients

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One of the ways to reduce chronic *P. aeruginosa* (Pa) infection in cystic fibrosis patients, is early treatment. This study evaluated both the infection-free period following successive episodes of colonization and treatment efficacy. Patients were treated with oral ciprofloxacin and nebulized colistin twice a day for the first isolation. If eradication failed, we continued to treat patients with oral ciprofloxacin and nebulized tobramycin for 28 days. Pa was considered eradicated after 3 consecutive negative respiratory cultures over 6 months. We treated a total of 88 episodes of first Pa colonization in 88 patients; 64 (84%) of the 88 episodes were treated with success, 12 (13.6%) failed. A second episode of colonization, after a mean infection-free period of 32.2 months, occurred in 49 (55.6%) out of 88 patients. Therapy was successful in 37 (75.5%) out of 49 patients, failed in 9 (18.3%). Furthermore 29 (32.9%) of 88 patients experienced a third colonization episode which occurred after a mean infection-free interval of 23 months. Therapy was successful in 19 (65.5%) out of 29 patients, failed in 6 (20.6%) but 15 (17%) of 88 patients experienced a fourth colonization episode after a mean infection-free interval of 18 months. Therapy was successful in 13 (86.6%) of 15 cases and failed in 2 (13.3%). There were no statistically significant differences in the number of successfully treated episodes according to different treatments. The infection-free period significantly decreased between colonization episodes ($p=0.02$). The percentage of success was not statistically different between episodes of successive colonizations. Early eradication treatment provided long infection-free periods although they became significantly shorter with recurring colonization.

74 Antibiotic resistance and hypermutator *P. aeruginosa*

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Introduction: Hypermutator *P. aeruginosa* constitutes an important part of the CF lung microbiology. Previous studies found that the presence of resistant mutant subpopulations (RMS: colonies growing in the zone of inhibition of an antibiotic disc on agar) can be used to test for them, and that they were associated with increased antibiotic resistance. We tested these findings in our adult CF patients.

Methods: 161 isolates from 40 patients were collected over 6 months. Hypermutators were detected according to previously published methods [1]. Each isolate had susceptibility testing (agar disc-diffusion method) to 9 antibiotics. The presence of RMS in 5 antibiotic zones was tested in a subset of 40 isolates.

Results: 47 (29.2%) isolates were hypermutators, and these were isolated from 16 (40.0%) patients. Mucoid isolates were more likely to be hypermutators than non-mucoid (42.5% vs 16.0%, $p<0.001$, χ^2), but hypermutator phenotype was not associated with small colony variants ($p=0.778$, χ^2) or the number of antibiotics (0–9) isolates were resistant to ($p=0.864$, Mann-Whitney). Hypermutators were more likely to have RMS to 3 or more antibiotics (45% vs 10%, $p=0.013$, χ^2). Receiver-operator curves for RMS as a test of hypermutator phenotype had area under the curve of 70%.

Conclusions: This is the first report of an association between hypermutator and mucoid phenotypes. Hypermutators were associated with the presence of RMS but, contrary to previous findings [1], the association with RMS was not specific enough to use it as a test for the hypermutator phenotype and hypermutators were not resistant to more antibiotics.

References

[1] Macia et al. Antimicrob Agents Chemother 2004; 48: 2665.

73* Occurrence of *P. aeruginosa* (PA) with hypermutable phenotype (HPM) in Italian CF patients

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Background: PA with HMP is associated with high antibiotic resistance rates in CF. AIM Determine occurrence and antibiotic resistance PA HMPs in Italian CF pts.

Methods: 154 non-mucoid, 100 mucoid and 18 SCV non-multiresistant PA from 170 pts from 4 Italian CF Centers were studied. HMP was determined as previously described (Macia MD) by disk-diffusion and Etest for 10 antibiotic; results were read after 24 h at 35°C; plates were re-incubated for further 12 h and HMPs were defined as strains showing a sub-population grown after 36 h and resistant to 3 or more antibiotics. The genetic relationship of all PA was determined by BOX-PCR. **Results:** 32% of pts was colonized with HMPs, mean age 28 yrs; pts without HMPs showed a mean age of 17 yrs. HMPs distribution was 17% in non-mucoid, 28% in mucoid and 39% in SCV strains. No correlation with HMP occurrence and shared genotypes was found. The % of antibiotic resistance in HMPs was AK 33, TO 12, CL9, FEP 51, CAZ 41, MEM 26, P/T42, T/C 47, CIP 35 and LEV 39.

Conclusion: The occurrence of 32% found, confirm previous rates reported from CF pts in other country (Oliver A.). The high occurrence found of in mucoid (28%) and SCV (39%) strains, typical in long term chronic colonization, stress the possible role of CF lung environment in inducing the expression of HMPs. In these strains the emerging resistance to TO and particularly to CL is worrisome for antimicrobial treatment implications.

Supported by: Italian Cystic Fibrosis Foundation Grant.

75 Persistence of metallo- β -lactamase-producing *Pseudomonas aeruginosa* in cystic fibrosis patients

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Introduction: Acquisition and spread of metallo- β -lactamases (MBLs), conferring resistance to nearly all β -lactams including carbapenems, is a matter of major concern. MBL-producing *Pseudomonas aeruginosa* strains are reported in hospital outbreaks worldwide, but very rarely from cystic fibrosis (CF) patients. The aim of this work was to assess the prevalence, persistence and genetic support of MBLs in *P. aeruginosa* from chronically colonized patients of CF Center of Florence and to analyse potential correlation with antibiotic treatment and patient outcome.

Methods: Forty carbapenem-resistant isolates of *P. aeruginosa* from 35 CF patients were assayed for MBL production by Etest and spectrophotometric assays. MBL genes and their genetic support were characterised by PCR and DNA sequencing. When MBL production was detected, multiple isolates from the same patient were retrospectively analysed. Clonality of the MBL-producing strains was investigated by RAPD.

Results: MBL activity was detected in 2 of 40 (5%) strains, obtained from 2 patients, which harboured the *bla*_{VIM-1} and *bla*_{VIM-2} gene respectively. Carriage of the same MBL variant by isolates from the same patient was detected over a prolonged period. Consecutive isolates either belonged in the same clone (for *bla*_{VIM-2}) or in 2 different clones (for *bla*_{VIM-1}). The 2 *bla*_{VIM} genes were carried on different class 1 integrons.

Conclusions: Two distinct variants of the *bla*_{VIM} resistance gene were detected in *P. aeruginosa* from CF patients over a prolonged period. The emergence of acquired MBL genes in *P. aeruginosa* in such patients is a phenomenon that could have important implications on surveillance protocols and clinical management.